

A spatial atlas of colorectal cancer reveals the influence of stromal niches on tumour differentiation

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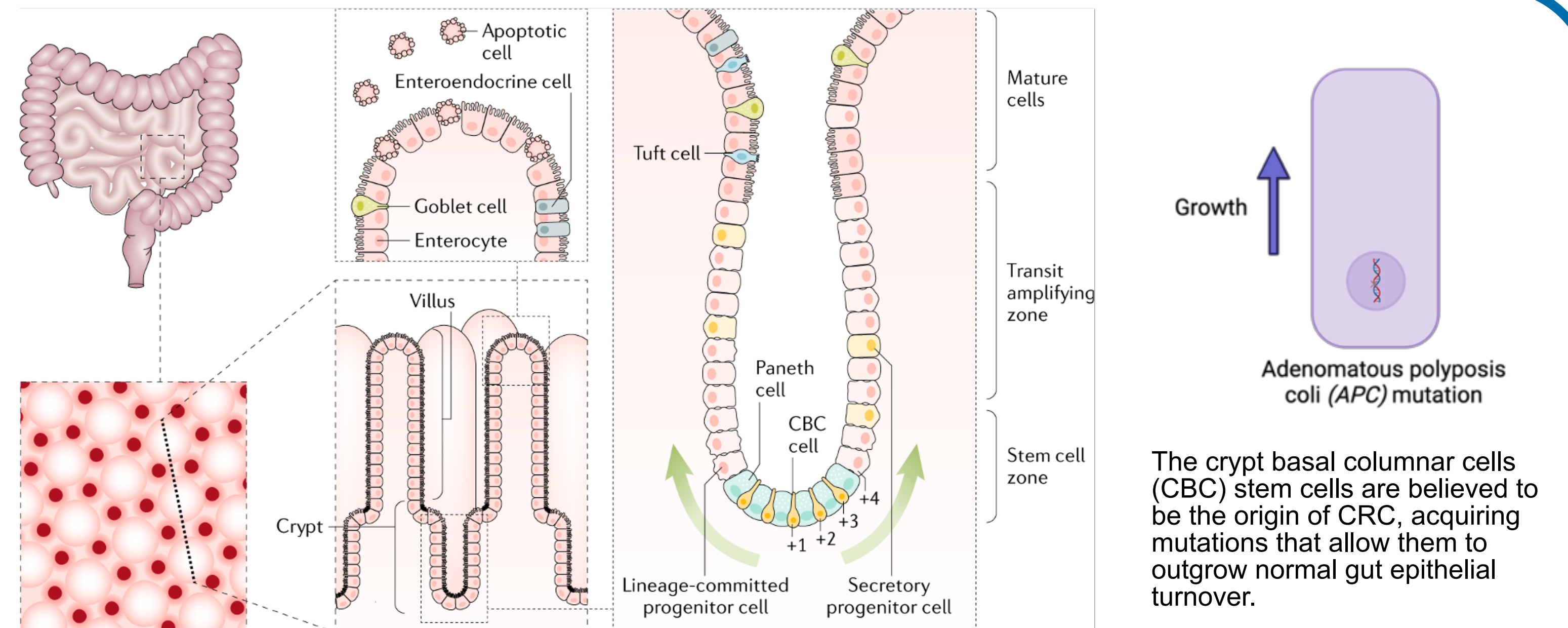
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Background

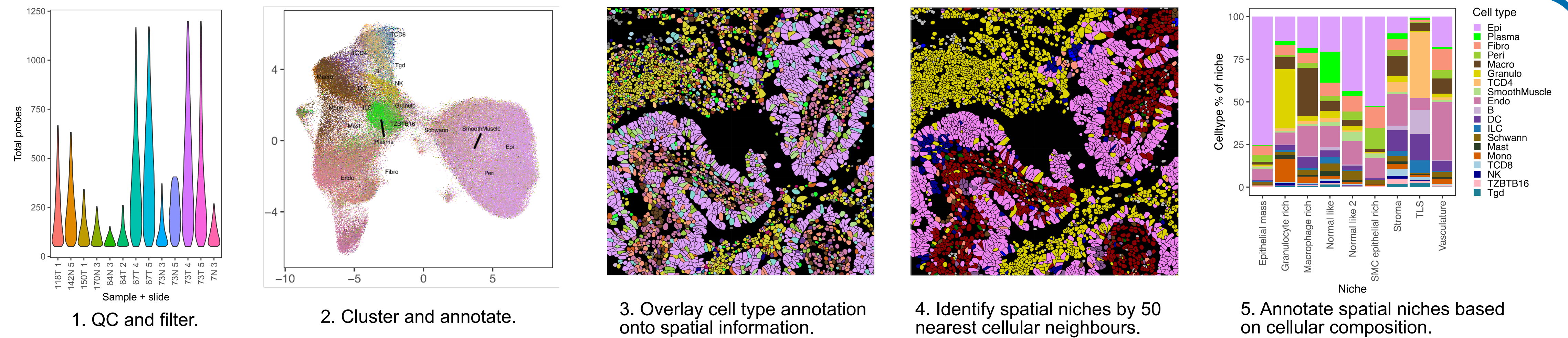
Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related mortality. Under healthy conditions, stromal scaffolds direct colonic epithelial cells to divide and differentiate from stem cells located at the base of the crypts to mature cell types which are constantly turned over. In contrast, tumour architecture is spatially heterogeneous, with the stromal tissues supporting the tumour epithelial cells either lost, altered or rearranged. To explore the spatial organisation of the tumour-immune microenvironment, we profiled the expression of ~1,000 genes in 846,469 cells from 23 tumour and normal colorectal tissue samples using the CosMx Spatial Molecular Imager (SMI).



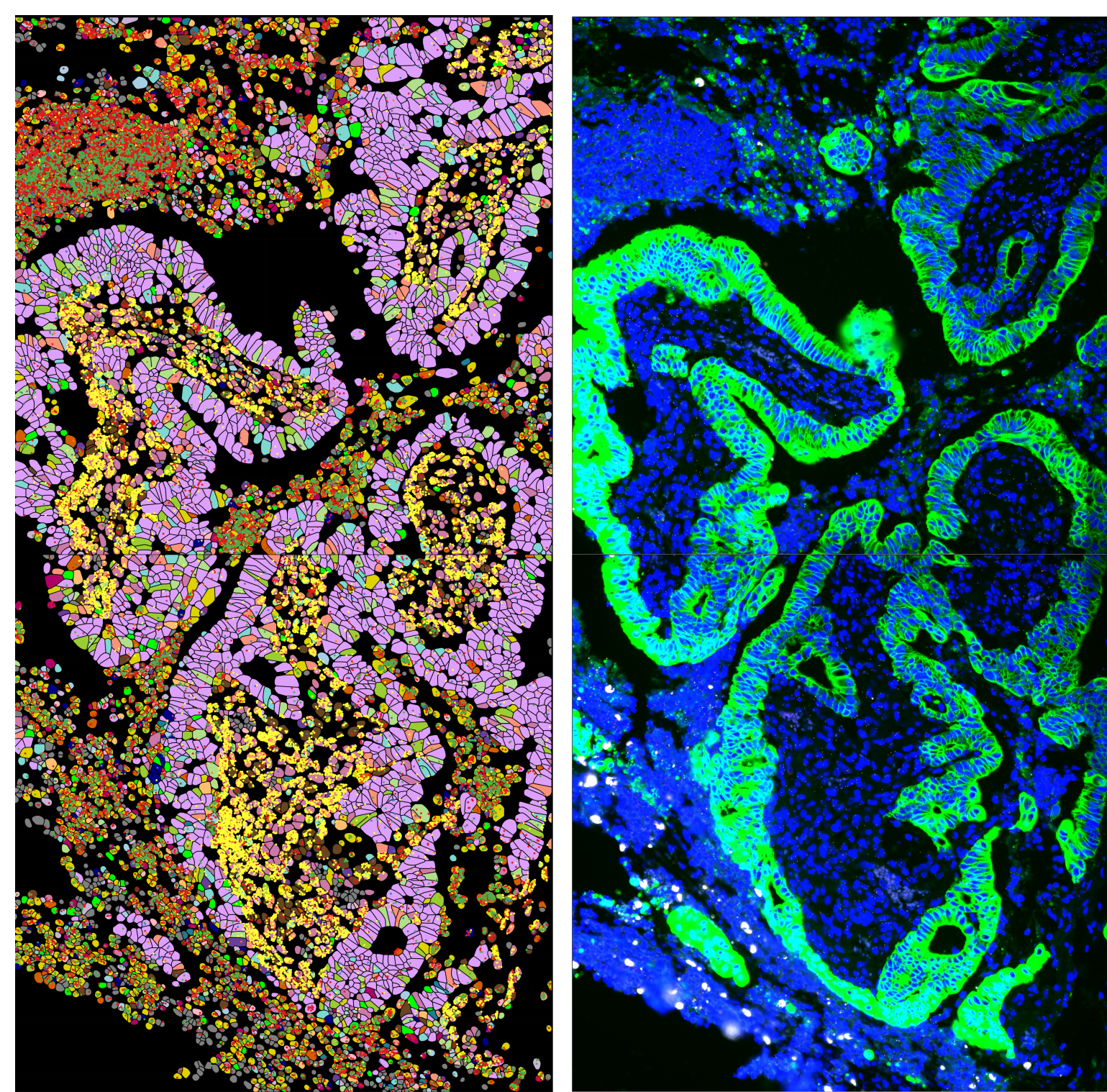
Renewal of the colonic crypt. Adapted from Gehart *et al.*¹.

Method

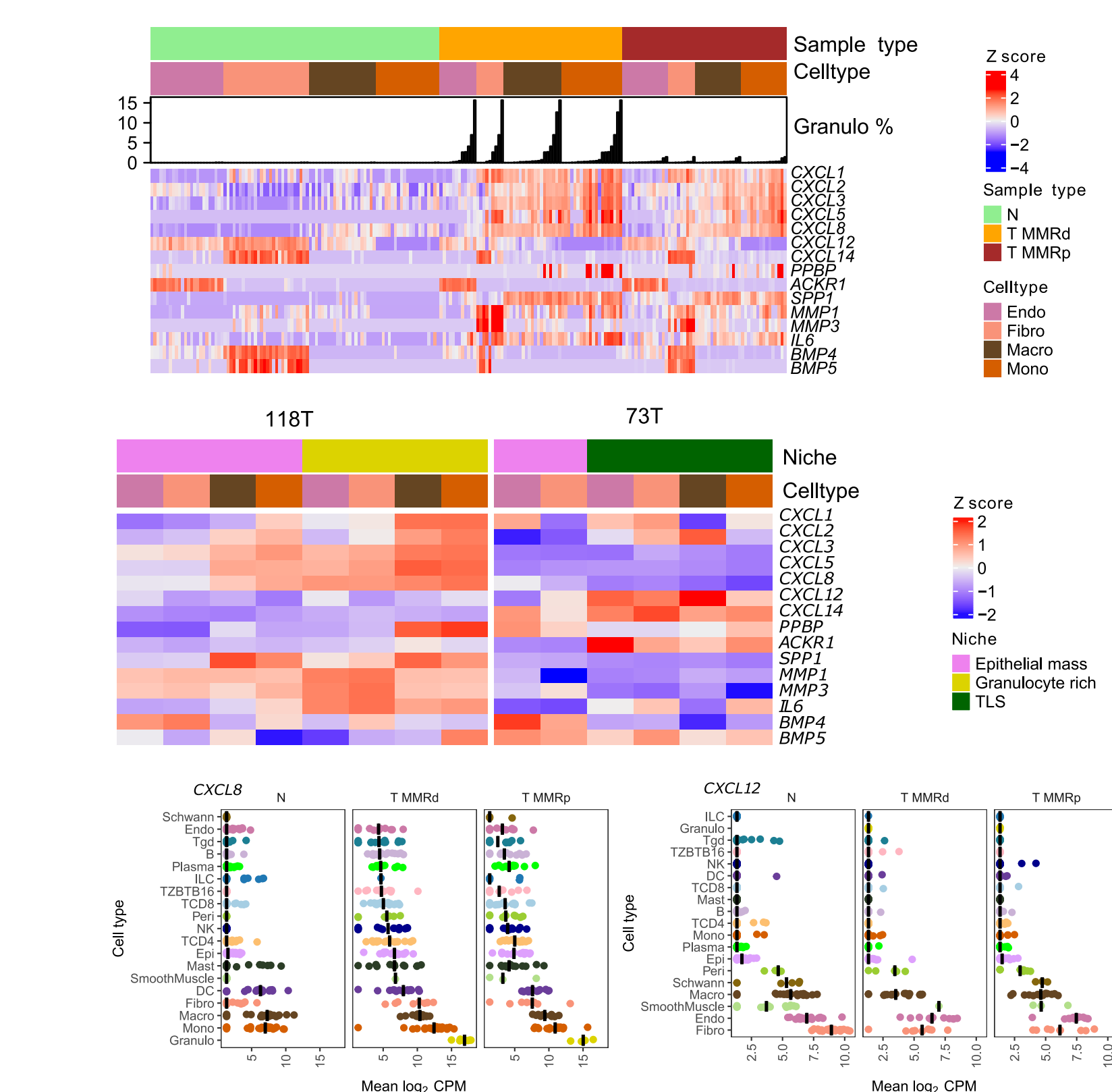
We measured the expression of 1000 genes in 846,469 cells from diverse tumour/normal tissues (23 samples, 15 donors) using the CosMx Spatial Molecular Imager. QC, cell type annotation and spatial niche identification were performed using a custom bioinformatic pipeline.



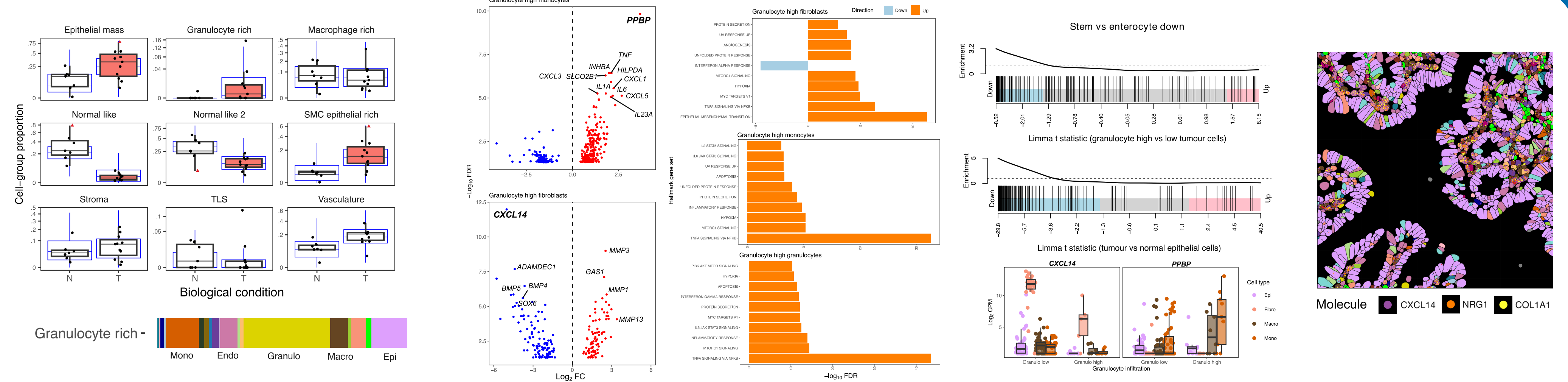
Results



CosMx analysis with seurat² revealed tumour regions with concentrated neutrophil infiltration.

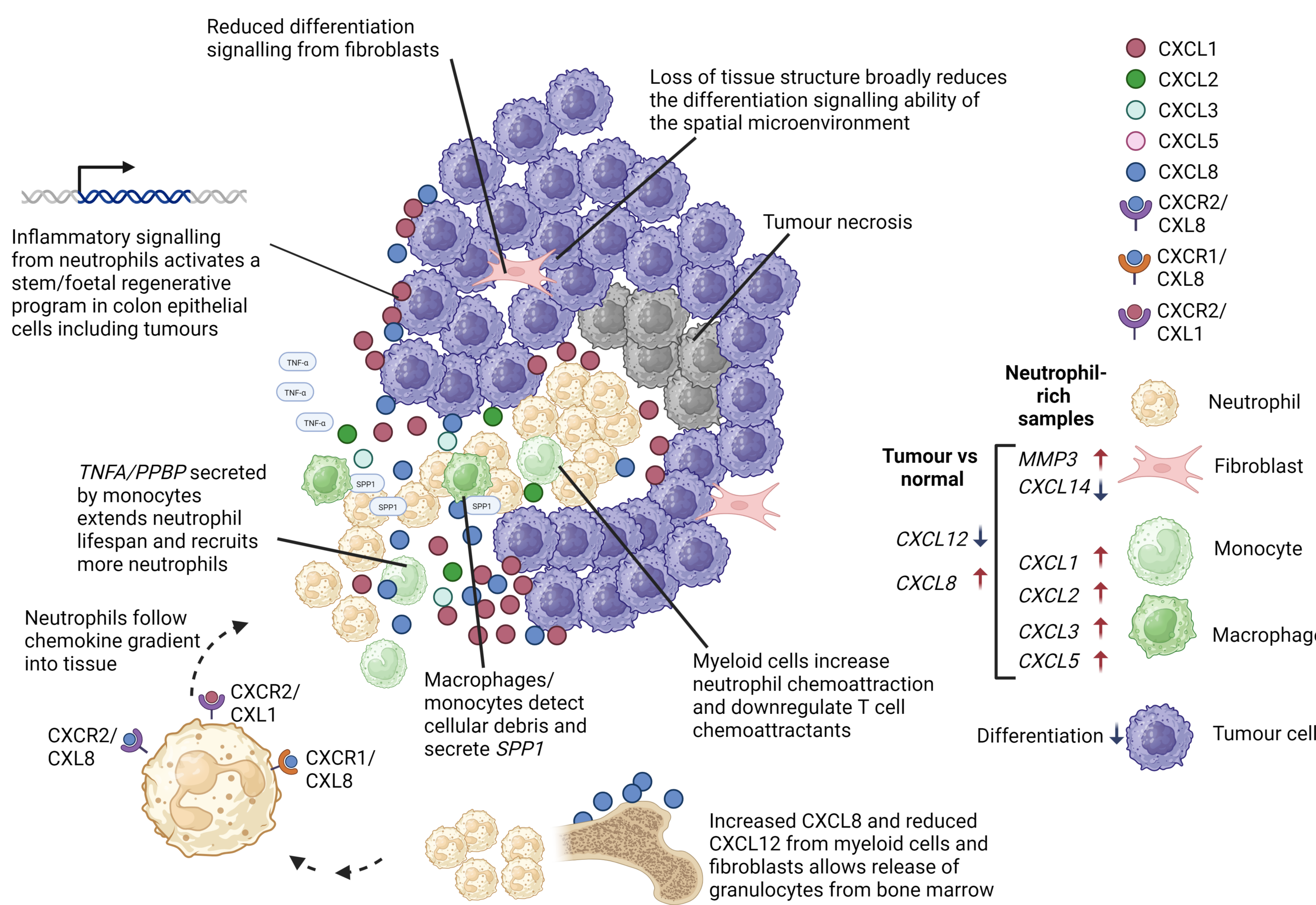


Tumours and tumour stroma broadly expressed neutrophil chemoattractants such as *CXCL8* and downregulated genes that prevent neutrophil release such as *CXCL12*⁵.

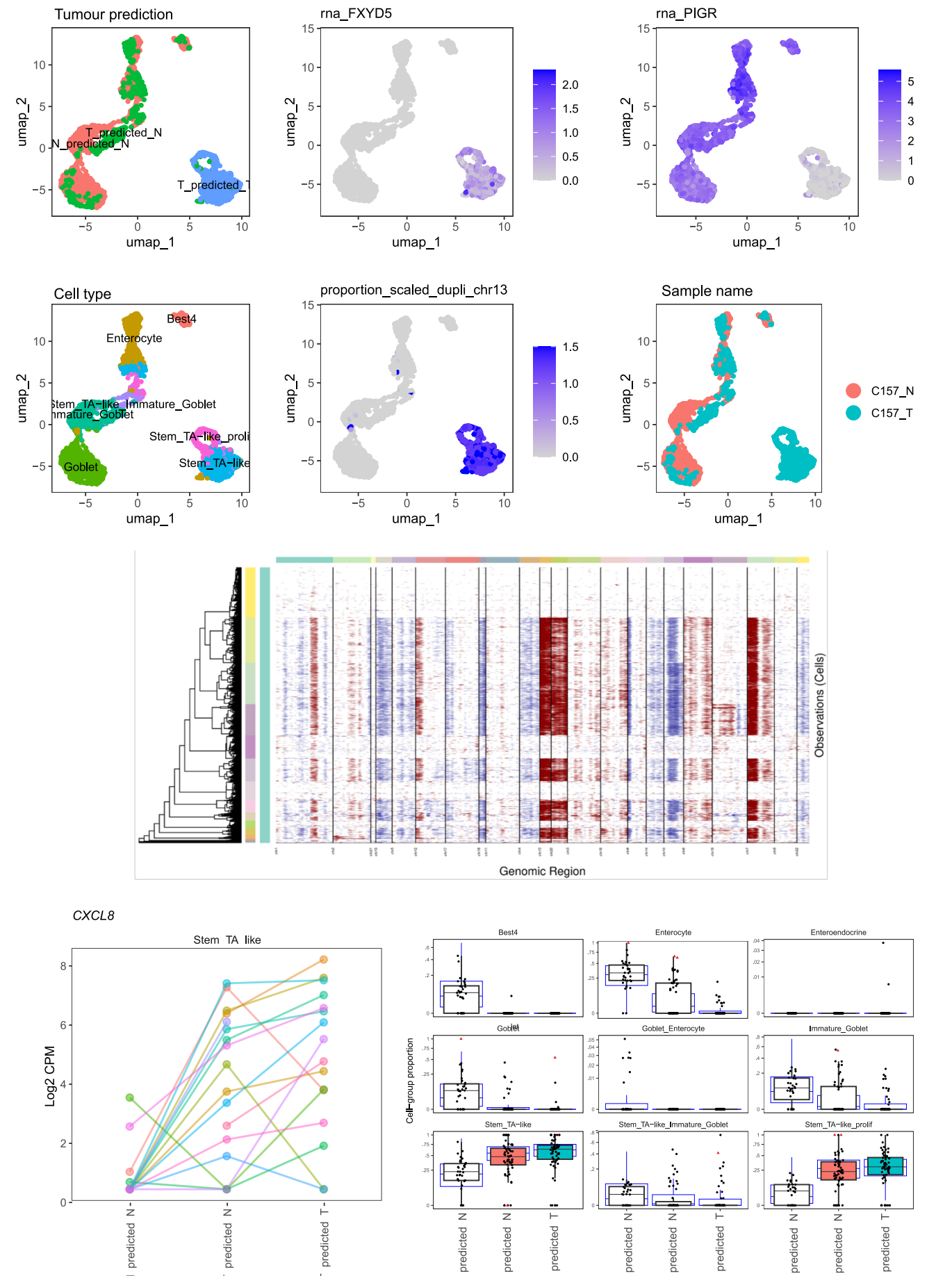


Spatial niche identification revealed 9 niches including a granulocyte rich niche. Sccomp³ showed that this niche is significantly enriched in tumours.

Splitting public scRNA-Seq samples⁴ into neutrophil high and low populations and performing pseudobulk DE with limma⁵ revealed increased inflammatory fibroblast and monocyte signalling. Furthermore, tumour cells were more stem-like and genes associated with the differentiation of epithelial cells in the normal colon (such as *CXCL14*) were down in fibroblasts, while genes associated with the activation of neutrophils (such as *PPBP*) were up in monocytes.



A model of neutrophil infiltration into necrotic CRC



Tumour cells could be identified, even within tumour samples, by the expression of dysadherin (*FXD5*) and the lack of polymeric immunoglobulin receptor (*PIGR*) expression (confirmed by inferCNV⁷ analysis). Comparing tumour and normal cells within tumours showed that neutrophil chemoattraction is a general response to necrosis and is not tumour intrinsic.

Conclusions

Neutrophil infiltration into necrotic tumours changes the stromal microenvironment.

Neutrophil infiltration aids in the maintenance of a stem-like phenotype.

Tumour cells are not directly responsible for neutrophil chemoattraction.

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- inferCNV of the Trinity CTAT Project. <https://github.com/broadinstitute/inferCNV>